CRP Ultra - C-Reactive Protein

For the Quantitative Determination of C-Reactive Protein (CRP) in Serum

Cat. No. KAI-060

INTENDED USE

For the quantitative determination of C-reactive protein in serum by latex particle enhanced immunoturbidimetric assay. Measurement of C-reactive protein aids in evaluation of the amount of injury to body tissues.

For In Vitro Diagnostic Use.

INTRODUCTION

C-reactive protein (CRP) is described in the literature as an acute phase protein that is involved in the activation of complement, acceleration of phagocytosis, and detoxification of substances released from damaged tissue. CRP is one of the most sensitive indicators of inflammation.

In response to an inflammatory stimulus, a rise in CRP may be detected within 6 hours. CRP is a sensitive, non-specific indicator of acute phase reactants. The level of CRP in serum is elevated in patients with arthritis or liver disease such as hepatitis A, hepatitis B, or biliary cirrhosis, and after severe infections such as septic shock.

The CRPUltra is intended for the quantitative determination of human CRP by latex particle enhanced immunoturbidimetric assay (ITA). ITA methods for quantitative determination of antibody and antigen immunoprecipitation complexes have been described.

PRINCIPLE OF TEST

Latex particles coated with antibody specific to human CRP aggregate in the presence of CRP from the sample forming immune complexes. The immune complexes cause an increase in light scattering which is proportional to the concentration of CRP in the serum. The light scattering is measured by reading turbidity at 572 nm. The sample CRP concentration is determined versus dilutions of a CRP standard of known concentration.

KIT COMPOSITION

Reagents (Liquid stable)

R-1: Buffer Reagent 1 x 30 ml
170 mM Glycine buffer solution

R-2: Latex Suspension 1 x 20 ml
0.17% (w/v) solution of latex particles coated with rabbit anti-human CRP antibodies

WARNINGS AND PRECAUTIONS

FOR IN VITRO DIAGNOSTIC USE.

Not to be used internally in humans or animals. Normal precautions for handling laboratory reagents should be followed.

Do not mix or use reagents from one test kit with those from a different lot number. Do not use reagents past their expiration date stated on each reagent container label.

Do not pipette by mouth. Avoid ingestion and contact with skin.

Reagents in this kit contain < 0.1 w/v% sodium azide as a preservative. Sodium azide may form explosive compounds in metal drain lines. When disposing of reagents through plumbing fixtures, flush with copious amounts of water.

REAGENT PREPARATION

Reagents are ready to use and do not require reconstitution. Mix gently before using.

STORAGE AND STABILITY

All reagents should be stored at 2-10°C and protected from light. Unopened reagents can be used for one year from the date of manufacture as indicated on the expiration date on the package and bottle labels. Once the reagent vial has been opened, store tightly capped at 2-10°C and use within 1 month.

INTERFERENCE

Hemoglobin, Lipid (Triglycerides), and Bilirubin do not interfere with the latex particle enhanced immunoturbidimetric determination of C-reactive protein.

Dust particles or other particulate matter in the reaction solution may result in extraneous light-scattering, which may affect the accuracy of this test.

SPECIMEN COLLECTION AND PREPARATION

Serum test samples must be collected in the manner routinely used for clinical laboratory tests. Freshly drawn serum is preferred and should be used within the day of collection. Samples may also be stored refrigerated (2-10°C) for one week or at -30°C for up to 1 year. Use undiluted samples for this assay.
Use plastic tubes for storing the sample, do not use glass.

AUTOMATED ANALYZER APPLICATION

Suitable for two-reagent automated analyzers that can measure a rate reaction at an absorbance at 572 nm. Refer to the instrument manual from the manufacturer regarding the following:

- Use or function
- Installation procedures and requirements
- Principles of operation
- Performance characteristics and specifications
- Operation instructions
- Calibration procedures including materials and/or equipment to be used
- Operational precautions, limitations, and hazards
- Service and maintenance

PROCEDURE

Materials Supplied

Reagent 1 (R-1) Buffer Reagent 1 x 30 ml
Reagent 2 (R-2) Latex Suspension 1 x 20 ml

Materials Required But Not Supplied

Multi-point calibrators:

Standard Protocol: CRPUltra Multi-Calibrator Set A, Catalog #KAI-061C, 5 calibrators; Approx. values: 50, 150, 500, 1000, 2000 µg/dl. (For actual values see Package Insert).

High Sensitivity Protocol: CRPUltra Multi-Calibrator Set B, Catalog #KAI-062C, 5 calibrators; Approx. values: 50, 150, 500, 750, 1000 µg/dl. (For actual values see Package Insert).

Automated chemistry analyzer: capable of accurate absorbance reading at 572 nm with appropriate cuvettes and calculating rate assays.

Isotonic saline

Pipettes: capable of accurately dispensing the required volumes

Test Tubes: glass or plastic

Assay Procedure

An example of standard protocol automated application:

Sample 6.0 µl

\[ \begin{align*}
\text{Sample} & \quad 6.0 \, \mu \text{l} \\
\downarrow & \\
\text{\textbullet} & \text{R-1 (Buffer Reagent)} \\
\downarrow & 37^\circ \text{C}, 5 \text{ min} \\
\text{\textbullet} & \text{R-2 (Latex Suspension)} \\
\downarrow & 37^\circ \text{C}, 1.6 \text{ min} \\
& \text{Rate for 1.5 min, 572 nm}
\end{align*} \]

Note: Allow all reagents and specimens to come to room temperature. Mix all reagents gently before using. Calibrator and samples are to be used undiluted.

Automated Method (Example)

Chemistry Parameters for Automatic Analyzer:

CRP Standard Parameter, Calibrator A

- Instrument: Hitachi 717
- Temperature: 37°C
- TEST (CRP 2)
- ASSAY CODE: (2 POINT):(28)-(43)
- SAMPLE VOLUME: (6)-(10)
- R1 VOLUME: (125) (20) (NO)
- R2 VOLUME: (125) (20) (NO)
- WAVELENGTH: (800)-(570)
- CALIB. METHOD: (NONLINEAR)(4)(6)
- UNITS: (µg/dl)
- STD.(1) CONC.-POS.: (0.0)-(1)
- STD.(2) CONC.-POS.: (50.0)-(2)
- STD.(3) CONC.-POS.: (150.0)-(3)
- STD.(4) CONC.-POS.: (500.0)-(4)
- STD.(5) CONC.-POS.: (1000.0)-(5)
- STD.(6) CONC.-POS.: (2000.0)-(6)
- SD LIMIT: (999)
- DUPLICATE LIMIT: (10000)
- SENSITIVITY LIMIT: (0)
- ABS. LIMIT (SLOPE): (32000)(INCREASE)
- PROZONE LIMIT: (-32000)(LOWER)
- EXPECTED VALUE: (-99999)(99999)
- PANIC VALUE: (-99999)(99999)
- INSTRUMENT FACTOR: (1.00)

Parameters for other automated analyzers are available.

Calibration Curve

It is recommended that a multi-point calibration curve be made using either the CRPUltra Multi-Calibrator Set A (standard protocol) or CRPUltra Multi-Calibrator Set B (high sensitivity protocol). Please be sure to use the proper instrument application for the calibrator you select. It is recommended that the user determine calibration frequency as this will depend on the instrument and type/number of other assays being run. Initially, calibration should be performed each day.

Quality Control

It is recommended that commercially available control serum with a known concentration of CRP be included in all assay runs.

RESULTS

Calculations

CRP levels are determined by the autoanalyzer using the prepared calibration curve.
LIMITATIONS OF PROCEDURE

Standard Protocol: The **CRP Ultra** has a measurable range from 10.0 to 2000 µg/dl (0.01 to 2.00 mg/dl) using the CRP Multi-Calibrator Set A and standard parameters.

High Sensitivity Protocol: The **CRP Ultra** has a measurable range from 5.0 to 1000 µg/dl (0.005 to 1.000 mg/dl) using the CRP Multi-Calibrator Set B and high sensitivity parameters.

Reagents should not be used after the expiration date indicated on the kit label. Do not mix reagents with different lot numbers.

If the CRP concentration is greater than highest calibrator value, dilute one part sample with four parts isotonic saline and re-assay. Multiply results by 5 to compensate for the dilution.

PERFORMANCE

The following performance data was obtained using a Hitachi 717 analyzer and standard protocol.

**Sensitivity**

When saline is used as a sample, the range of absorbance change per minute is -0.0050 to 0.0050, while a standard CRP solution containing 1000 µg/dl is 0.0650 to 0.1000 after subtracting the saline blank.

**Specificity**

When serum containing a known level of CRP (250 µg/dl) is measured, the assay value obtained is within ±10%.

**Precision**

Samples tested were commercial human CRP control serum.

**Precision Assay:**

(Within-run)

<table>
<thead>
<tr>
<th>Sample I</th>
<th>Sample II</th>
<th>Sample III</th>
</tr>
</thead>
<tbody>
<tr>
<td>N=20</td>
<td>N=20</td>
<td>N=20</td>
</tr>
<tr>
<td>Mean=2.74 mg/L</td>
<td>Mean=8.64 mg/L</td>
<td>Mean=17.43 mg/L</td>
</tr>
<tr>
<td>SD=0.034</td>
<td>SD=0.068</td>
<td>SD=0.135</td>
</tr>
<tr>
<td>CV=1.24%</td>
<td>CV=0.78%</td>
<td>CV=0.77%</td>
</tr>
</tbody>
</table>

**Precision Assay:**

(Between-run)

CRP values were tested on 3 days.

<table>
<thead>
<tr>
<th>Sample I</th>
<th>Sample II</th>
<th>Sample III</th>
</tr>
</thead>
<tbody>
<tr>
<td>N=10</td>
<td>N=10</td>
<td>N=10</td>
</tr>
<tr>
<td>Mean=2.65 mg/L</td>
<td>Mean=8.55 mg/L</td>
<td>Mean=17.13 mg/L</td>
</tr>
<tr>
<td>SD=0.111</td>
<td>SD=0.092</td>
<td>SD=0.204</td>
</tr>
<tr>
<td>CV=4.2%</td>
<td>CV=1.08%</td>
<td>CV=1.19%</td>
</tr>
</tbody>
</table>

**Correlation**

y= 1.034x - 0.173

r= 0.999

x= Company A’s latex CRP nephelometric assay

y= **CRP Ultra**

INTERFERENCE

- Bilirubin C: No interference up to 60 mg/dl.
- Bilirubin F: No interference up to 60 mg/dl.
- Hemoglobin: No interference up to 500 mg/dl.
- Lipid: No interference up to 1500 mg/dl triglycerides.

EXPECTED VALUES

Expected value for CRP in healthy individuals is from 0.007 to 0.494 mg/dl. This value was calculated using 496 healthy adults. It is recommended that each laboratory establish its own expected range.

REFERENCES


ORDERING/PRICING/TECHNICAL INFORMATION:

Manufactured for:

EQual Diagnostics, Inc.
115 Summit Drive
Exton, PA 19340

TEL: 800-999-6578
FAX: (610) 594-8585

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